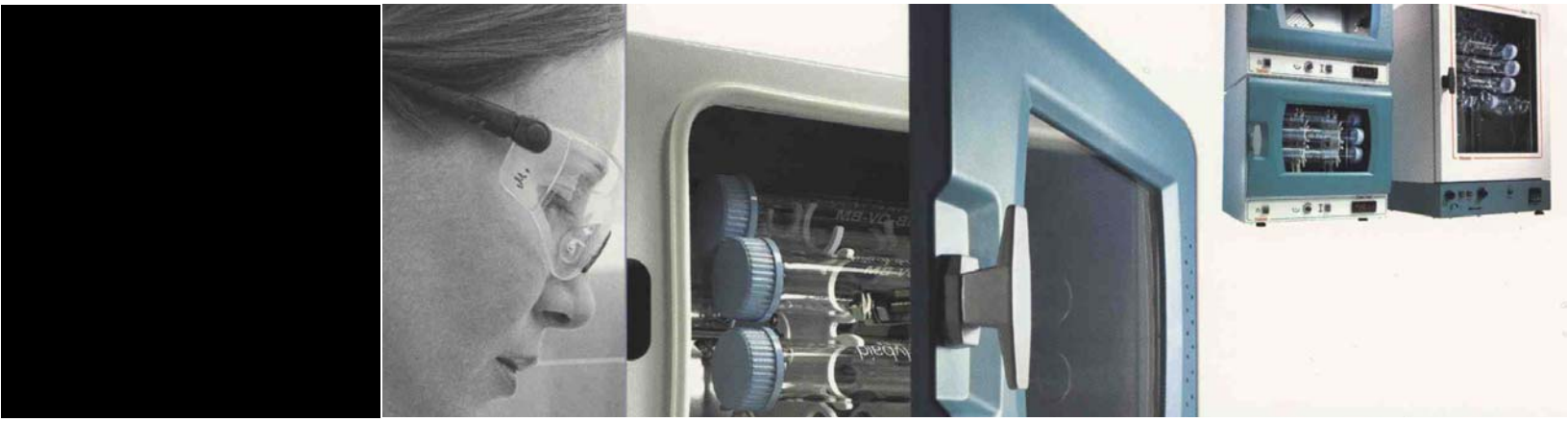
Maxi 14

Hybridisation Oven

Operating and Maintenance Manual 7006246 Rev. 4



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Models Covered:

Model	Voltage	Rotisserie
6246	110V	1.4 inch (35mm)
6247	220V	1.4 inch (35mm)
6248	110V	2.8 inch (70mm)
6249	220V	2.8 inch (70mm)

MANUAL NUMBER 7006246

REV	ECR/ECN	DATE	DESCRIPTION	By
4	25799/OV-325	10/28/09	Added caution not to over-tighten lid of bottle	ccs
3	24622/OV-292	5/15/08	updated elecschematics to show country dependent line cord	ccs
2	23459	6/12/06	Updated bottle to 3.2 wall thickmness, added X-ray warning	ccs
1	--	12/2/03	Added Model 6248, 6249, new rotisserie 70mm	ccs
0	--	--	Original	ccs

Important Read this instruction manual. Failure to read, understand and follow the instructions in this manual may result in damage to the unit, injury to operating personnel, and poor equipment performance. ▲

Caution All internal adjustments and maintenance must be performed by qualified service personnel. ▲

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Important operating and/or maintenance instructions. Read the accompanying text carefully.

Potential electrical hazards. Only qualified persons should perform procedures associated with this symbol.

Equipment being maintained or serviced must be turned off and locked off to prevent possible injury.

Hot surface(s) present which may cause burns to unprotected skin, or to materials which may be damaged by elevated temperatures.

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- ✔ Always dissipate extreme cold or heat and wear protective clothing.
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Regardless of your needs, our professional telephone technicians are available to assist you Monday through Friday from 8:00 a.m. to 6:00 p.m. Eastern Time. Please contact us by telephone or fax. If you wish to write, our mailing address is:

Thermo Scientific
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401 Millcreek Road, Box 649
Marietta, OH 45750

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Section 1 Introduction

The Thermo system of hybridising in bottles is considered by many molecular biologists to be the best method for conducting hybridisations with Southern, Northern, Dot, Slot or Colony Blots. Additional information, including detailed protocols, is contained in the Hybridisation Guide.

Hybridising in bottles means that probe volumes may be significantly reduced compared to experiments performed in conventional systems and the continual movement of the probe across the surface of the membrane results in very efficient hybridisation reactions.

Protection from exposure to radiation is provided by the heavy walled borosilicate glass bottles.

Warning Based on tests, these bottles block almost 100% of BETA radiation emissions of a 1,000 pCi P-32 source. However, users should observe precautions due to induced X-ray phenomenon (Bremsstrahlung) occurring, depending on the type(s) of radionuclides used. ▲

The Maxi 14 Hybridisation Oven itself provides additional shielding. In the event of a spillage within the Oven, the stainless steel drip tray will contain up to 6.7 fl. oz. (200ml) of liquid.

Oven Temperature Monitoring

All Ovens are calibrated by matching the thermistor temperature read-out, seen on the LCD display, to that actually measured in fluid contained within a Hybridisation Bottle as it rotates. The thermometer used inside the calibration bottle is traceable to national standards.

This calibration method ensures all experiments carried out using the rotisserie, meet our temperature accuracy specification of ±1°C and indeed due to the rotisserie action, uniformity within the bottle is ±0.25°C. However, it is important to note that the temperature inside the oven chamber will vary from point to point, therefore, the temperature of objects/vessels, which are not placed in the rotisserie, will vary from that which is displayed.

Oven Temperature Monitoring (cont.)

The Maxi 14 Hybridisation Oven is supplied with either two or four hybridisation bottles. These high quality leak-proof containers can be used at temperatures up to 70°C (for part numbers, see Chapter 8).

Bottles should not be used above 70°C without taking the precaution of releasing the pressure formed by the release of gases from solutions as they are warmed. The pressure should be relieved by simply loosening and then retightening the bottle cap at approximately 60°C.

Caution Dropping or over-tightening lids can cause cracking and failure. Lids should be inspected prior to each use. ▲

Note Remember to always wear gloves when handling the bottle and to use the bottle gripper provided (see Appendix II for full instructions on Bottle Care). ▲

Finally, the Maxi 14 Hybridisation Oven has been designed for reliability and for ease of maintenance. Hence, the rotisserie, drip tray/shaking platform can be easily removed for cleaning.

Thermo offers full service and technical support for all its products.

Section 2 Unpacking and Installation

Maxi 14 Oven Packing List	
Maxi 14 Oven	Orbital platform
Power cable	Linear platform
1.4" (35mm) rotisserie	Leveling feet
Drip tray	Hybridisation Guide
4 medium sized bottles	Bottle gripper
1 pack mesh	

If any item is missing or damaged, contact the Technical Services Department, or an authorised Thermo distributor.

Warning If it is intended to use the Oven with radioactive isotopes, it must be located in a designated Radiation Area. Radiation safety procedures must be followed at all times. In the event of a spillage, refer to Chapter 6 for guidance on cleaning or decontamination.

Maxi 14 Hybridisation Oven standard rotisseries are designed to accept our long, medium or short hybridisation bottles; extra rotisseries are available to accept extra long bottles.

Once unpacked, attach leveling feet to base, and position on a flat surface. Level the Oven by adjusting the height of the leveling feet. Once leveled, the Oven is ready for use.

Connect the Oven to the mains power supply using only the removable mains line cord supplied. The line cord should be fitted with a 10A fuse for both 110/120V and 220/240V applications. The Oven is grounded via the mains line cord and should only be connected to an grounded supply. See Specifications.

Operation

The three switches found on the control panel operate the power, rotisserie and shaking platform. The power switch is marked with a “1” and a “0”. By switching to the “1” position, the mains power is connected to the Oven and a green section of the switch is exposed to indicate that the Oven is “ON”. The switches to the left operate the shaking platform and the rotisserie. The rotisserie and shaking platform switches cannot be operated without first turning on the power switch.

Instructions on how to set up the shaking platform are given in Chapter 4.

Setting the Required Temperature

The Maxi 14 Hybridisation Oven is fitted with a Type II temperature controller. Follow the instructions below to set the temperature.

Type II temperature controllers have 3 buttons marked “★”, “▲“ and “▼“ underneath the temperature display.

To reset the temperature, press the key marked “★” while pressing either the up or down arrow key until the desired temperature is shown in the display, then release the “★” button. If the button marked “★” is pressed and released, the temperature display will show the programmed temperature setpoint.

The warm-up time for the Oven varies according to the load and the ambient temperature. When first turning on the Oven, allow approximately one hour for the temperature to stabilise. Solutions should be pre-warmed to the required temperature to minimise sample stabilisation time.

The lowest target temperature which the Oven can achieve is 8°C above ambient temperature. Avoid placing the unit in direct sunlight or in any area where it is likely to be exposed to hazardous or volatile chemicals.

When positioning the Maxi 14 Hybridisation Oven, ensure a gap of at least 2 in. (5cm) is maintained between the sides of the Oven and any other equipment or object.

Setting the Required Temperature (cont.)

The Maxi 14 Hybridisation Oven may be located in a fume cabinet or similar chamber, provided the cabinet is not used to contain hazardous or volatile chemicals with low flash points.

Full details of Technical Specifications are given in Chapter 8.

Note If the Maxi 14 Hybridisation Oven is not used as specified in this manual, the protection provided by the equipment may be impaired. ▲

Section 3 Methodology

The following paragraphs detail the methodology of hybridisation using the Maxi 14 Hybridisation Oven.

Placing Membranes in a Bottle

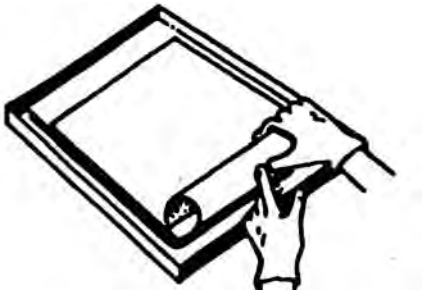
1. Place the bottles to be used in the Oven during the warm up period (approximately 1 hour).
2. Select a piece of support mesh appropriate for the size of the membrane. The recommended hybridisation mesh is supplied by Thermo (see parts list in Chapter 8).
3. Pre-wet the mesh and hybridisation membrane in a suitable tray containing 2 x SSPE (SSC) (see Figure 3-1).
4. Ensuring that the hybridisation membrane exactly overlays the mesh, roll both up into a tight roll.

   If more than one membrane is to be hybridised in a bottle, simply overlay further meshes and membranes as required before rolling. It is important that each membrane is separated from any other by a piece of mesh. Up to five 0.8 x 0.8 in. (20 x 20mm) membranes can be hybridised in a single hybridisation bottle.
5. Place 0.34-0.51 fl. oz. (10-15ml) SSPE (SSC) into a hybridisation bottle, then insert the roll in such a way that the leading edge (inside the roll) and the trailing edge are positioned relative to each other (as shown in Figure 3.3).
6. Place the bottle on a flat surface, then slowly unwind the membrane and mesh around the inside of the bottle by rocking and gently rolling the bottle along the surface. No air bubbles should be visible between the membrane and the bottle. If bubbles are present, the membrane should be removed and re-rolled.

   The procedure should then be repeated more gently. Rock the bottle backward and forwards to attach the first part of the membrane to the bottle. Then roll the bottle in order to unwind the mesh and membrane.
7. Continue until the membrane and mesh are fully unwound. The mesh ensures the probe will have access to all parts of the hybridisation membrane including those parts of the membrane which overlap.

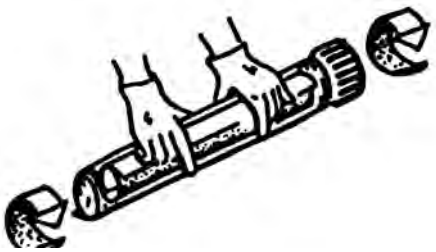
Insertion

1. Needed: Shallow tray containing 2 x SSPE (SSC), mesh, membrane.



2. Roll up mesh & membrane.

3. Place 10-15mls of SSPE (SSC) into a bottle, then insert the roll centrally.



4. Secure cap and, holding bottle horizontally, roll to catch the trailing edge of the mesh, continue rolling in the same direction until coil of mesh and membrane is well positioned.

5. Pour out SSPE (SSC) and replace with pre-warmed pre-hybridisation buffer.

6. Place the bottle in the Oven so that it rotates in the Oven in the same direction it was unrolled in Step 4.

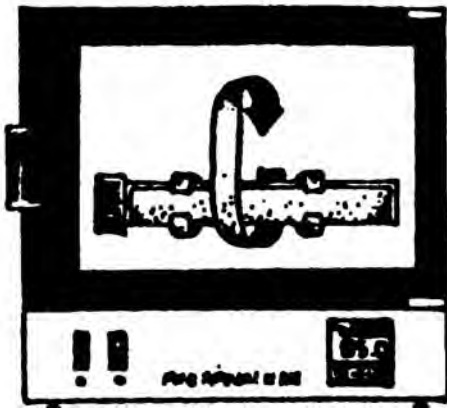


Figure 3-1. Insertion

Pre-Hybridisation

1. When the membrane is in place in the bottle, the SSPE (SSC) can be discarded and replaced with pre-hybridisation fluid.

   This is easily done by removing the cap, pouring off the SSPE (SSC), then pouring in the pre-hybridisation fluid. All the solutions used during hybridisation should be pre-warmed before use. 0.34-.51 fl. oz (10-20ml) of solution is recommended for medium bottles and 0.17-0.34 fl. oz. (5-10ml) for short bottles. Higher volumes will be required if there is more than one membrane in the bottle, e.g. 0.51-0.85 fl. oz. (15-25ml).

2. Replace the cap on the bottle and insert into the rotisserie.

Hybridisation

1. Denature the purified probe by boiling for five minutes, then store on ice. If the volume to be used for hybridisation is substantially different to that used previously for hybridisation in bags or boxes, ensure that the quantity of the probe is adjusted accordingly to maintain the correct probe concentration. If this is not done, high background may result.

2. Remove the bottle from the Maxi 14 Hybridisation Oven and unscrew the cap.

3. If the same buffer is to be used for hybridisation as for pre-hybridisation, simply pipette the probe into the pre-hybridisation fluid in the bottle. Take care to avoid pipetting the probe directly onto the membrane as this will result in hot spots. Alternatively, dilute the probe in pre-warmed hybridisation solution outside the bottle. Pour off the pre-hybridisation solution and replace with the probe solution.

4. Replace the cap and gently agitate the bottle to ensure an even distribution of the probe in hybridisation solution.

5. Place the bottle back in the Oven, switch on the rotisserie and allow it to hybridise for the required time period.

Washing

There are two methods of washing: bottle or box. Details follow.

Method 1 - Washing in Hybridisation Bottles

1. Remove the bottle from the Oven.
2. Pour off the hybridisation fluid, then half-fill the bottle with the first pre-warmed wash solution (approx. 3.38 fl. oz. [100ml]) and replace the cap.

Note Room temperature washing or washing with solutions which have not been pre-warmed may result in background problems (see Appendix I of the Hybridisation Guide). ▲

3. Replace the bottle in the Oven and leave for the required time period.

   If transferring from sandwich box or plastic bag methodology, the times and temperatures are usually those which are currently being used, approximately 20 minutes per wash step.

4. Repeat Steps 1-3 for each additional wash. The wash solutions, temperatures, etc., should be those recommended by the membrane manufacturer, or refer to the Hybridisation Guide.

Note All wash solutions should be pre-warmed for best results. ▲

Method 2 - Washing in a Sandwich Box

Some scientists prefer to remove the membranes from the bottles and wash them all in one container. Washing may be performed very simply on the shaking platform and is particularly useful when several membranes are being processed simultaneously.

1. Remove the hybridisation bottle from the Oven. Pour off the hybridisation fluid.
2. Using a pair of forceps, carefully remove the membranes and place them in a suitable container with a lid (e.g. sandwich box or similar).
3. Using the forceps gently unroll the membranes.

(continued)

Method 2 - Washing in a Sandwich Box (cont.)

4. Add a sufficient volume of the pre-warmed first wash solution to totally immerse the membranes. Replace the lid and shake on the linear motion shaking platform in the Maxi 14 Hybridisation Oven at the required temperature for the specified time period. Initial room temperature washes are not recommended and may result in subsequent background problems.

5. Remove the first wash solution and replace it with an equal volume of the pre-warmed second wash solution. Replace the lid and shake at the required temperature for the required time period.

6. Step 5 should be repeated for any subsequent wash. The washing protocol is obviously the same as is currently being used.

Other Procedures

In addition to bulk processing of blots during stringency washing steps, the shaking platform is ideal for performing several other stages of the blot generation and processing procedures. Three examples are given as follows:

1. Pre-treatment of Gels prior to Nucleic Acid Transfer

   Gentle agitation of the gel during pre-treatment steps is essential to prevent damage to the gel.

   Pre-treatment steps are usually performed at ambient temperature.

   (i) Depurination - 0.25M HCl 10 minutes

   (ii) Denaturation - 1.5M NaC1, 0.5M NaOH, 30 minutes

   (iii) Neutralisation - 1.5M NaC1, 0.5M Tris CI pH 7.2, 30 minutes

2. Pre-washing of Filters prior to Colony Blot Hybridisation

   Pre-washing is often advantageous to remove colony debris and hence reduce background noise when screening bacterial colonies by hybridisation. After lysing the bacterial colonies and fixing the DNA on the membrane, pre-wash in a solution of 2 x SSC/0.1% SDS at 65°C. Use several changes of buffer.

   After several washes, residual colony debris may be removed by gently rubbing filter with a gloved finger.

3. Probe Stripping from Filters after Hybridisation

   An example of probe stripping procedure is as follows:

   Wash the filter at 65°C in 5mM Tris Cl pH8.0, 0.2mM EDTA, 0.1 x Denhardt's reagent for 1-2 hours.

   Check for residual activity by autoradiography, then re-probe.

Section 4 Shaking Incubations

It is possible to perform shaking incubations with the Maxi 14 Hybridisation Oven.

1. Orbital Motion

   For orbital shaking, select the shaking platform with the round plastic central piece and four pegs. Ensure that the label faces towards the front of the Oven. The hole in the central plastic piece fits on the drive pin and the four pegs locate in the outer channels of the four plastic support pillars in the base of the unit.

2. Linear Motion

   For linear shaking motion, select the shaking platform with the rectangular central piece and four metal boards. The hole in the central piece fits on to the drive pin in the base of the unit with the hole pointing from front to back.

After the appropriate platform has been fitted, switch on the shaking platform and select the desired speed.

Section 5 Rotisseries

All Maxi 14 Hybridisation Ovens are equipped with the variable axis rotisserie. This allows the user to alter the angle at which bottles can be held in the rotisserie. A small offset from horizontal of between 1-15° causes fluid to flow from end to end within the bottles during rotation (see Figure 3). This "wave" motion provides a more vigorous and active hybridisation or washing stage and can allow probe volumes to be reduced to as low as 0.07-0.17 fl. oz. (2-5ml). To achieve the optimum active wave conditions requires adjustment of the rotisserie angle. Note that a large angle offset from horizontal (15°) combined with a low probe volume (<0.03-0.07 fl. oz. [1-2ml]) can produce dry areas on the membrane and so should be avoided.

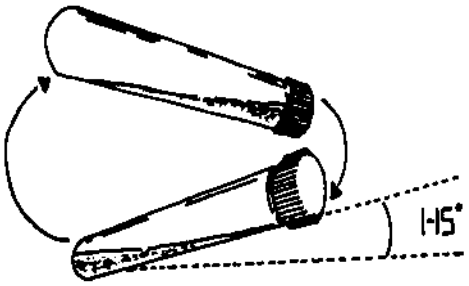


Figure 5-1. Variable Axis Rotation

To alter the bottle angle:

1. Release the two securing screws visible on the front of the rotisserie wheel using a Phillips screwdriver.
2. Put the rotisserie into the Oven and place a bottle in the rotisserie.
3. Rotate the free wheel to the desired angle.
4. Remove rotisserie and tighten the screw in the new position.

1.7 & 0.5 fl oz Tube Rotisseries

Rotisseries are available that can hold 0.5 fl. oz. (15ml) and/or 1.7 fl. oz. (50ml) tubes. Details are given in Chapter 8.

Caution Bottles should be inserted into the rotisserie by sliding the bottle in sideways. Care should be taken not to push the bottles too firmly into the rotisserie as the bottles will crack. ▲

Section 6 Cleaning and Decontamination

All items which come into contact with a labelled probe should be decontaminated before re-use. In many cases, a short rinse in water will be sufficient. In some instances, however, further decontamination procedures will be required.

Mesh

The quantity of radioactivity adhering to the mesh can be minimised by leaving the mesh in with the membrane throughout the washing procedure. Any radioactivity remaining can then be stripped from the mesh by the following procedure:

1. Strip wash the mesh by incubating it in distilled water at 65°C in a shaking water bath for 15 minutes. Repeat.
2. If the mesh is still contaminated, soak it in a diluted solution, such as Decon 90, overnight.
3. Remove the detergent and proceed with two washes in distilled water for 10 minutes each.
4. If the mesh has been decontaminated, no further action is necessary. If, however, the mesh is still contaminated, it will be necessary to leave it to decontaminate in Decon 90 for a longer time period.
5. Mesh should be allowed to dry flat between uses.

Bottles and Caps

The hybridisation bottles and caps can be decontaminated by the following procedure:

1. Soak the bottles and caps in a diluted detergent solution (Decon 90) overnight.
2. Remove the Decon 90 and rinse with distilled water.
3. If the bottles and caps have been decontaminated, no further action is necessary. If, however, they are still contaminated, gently scrub them with an abrasive cloth or brush and, if necessary, leave to decontaminate in Decon 90 for a longer time period.

Oven Interior/Shaking Platform

Stainless steel drip trays are provided with all Ovens and are intended to contain spillages in the event of an accident. These, together with the stainless steel surfaces of the Ovens and the shaking platform, can be decontaminated by wiping clean with Decon 90, followed by distilled water rinse. No further action should be necessary.

Rotisserie

The rotisserie may be cleaned with Decon 90 and distilled water. No further action should be necessary. Avoid alcohol or other organic solutions, which may affect the plastic surfaces.

Section 7 Use of Radioactive Probes

The Maxi 14 Hybridisation Oven has been tested with a variety of non-radioactive probes, e.g. the DIG Non-Radioactive Nucleic Acid labelling and detection system from Boehringer Mannheim.

Refer to the Hybridisation Guide for guidelines on the use of non-radioactive systems.

Warning Refer to notes on the use of hazardous chemicals in the Warranty section. ▲

Section 8 Specifications

Description	**Maxi 14**
110V	Model 6246
220V	Model 6247
Temperature	
Range	+8°C above ambient to +85°C
Selection	Digital
Display	LED
Uniformity (within the bottle)	±0.25°C
Accuracy (at 55°C)	±1.0
Safety	
Overtemp Cut-out	Yes
Rotisserie	
Bottle capacity	14
Material	Stainless steel shaft, variable axis 0-15°, stainless steel rotisserie wheels
Variable speed rotisserie	Yes
Rotisserie speed	5-15 RPM
Shaking Platform	Yes
Shaking action	Orbital and linear
Shaking platform area	13 x 12" (330 x 300mm)
Maximum weight	8.8 lbs. (4kg)
Maximum power	250W
Dimensions	
External	18"W x 28"H x 18" (450mmW x 690mmH x 456mmD)
Internal	13.8"W x 20.1"H x 12.6"D (350mmW x 510mmH x 320mmD)
Shipping Weight	110 lbs. (50kg)
Standard Accessories	1.4 in. (35mm) rotisserie
	Drip tray
	Four medium bottles
	1 pack mesh
	Large bottle gripper
	Orbital platform
	Linear platform

Description	**Maxi 14**
110V	Model 6248
220V	Model 6249
Temperature	
Range	+8°C above ambient to +85°C
Selection	Digital
Display	LED
Uniformity (within the bottle)	±0.25°C
Accuracy (at 55°C)	±1.0
Safety	
Overtemp Cut-out	Yes
Rotisserie	
Bottle capacity	6
Material	Stainless steel shaft
	Stainless steel rotisserie wheels
	Variable axis 0-15°
Variable speed rotisserie	Yes
Rotisserie speed	5-15 RPM
Shaking Platform	Yes
Shaking action	Orbital and linear
Shaking platform area	13 x 12" (330 x 300mm)
Maximum weight	8.8 lbs. (4kg)
Maximum power	250W
Dimensions	
External	17.7"W x 27.2"H x 17.9"D (450mmW x 690mmH x 456mmD)
Internal	13.8"W x 20.1"H x 12.6"D (350mmW x 510mmH x 320mmD)
Shipping Weight	110 lbs. (50kg)
Standard Accessories	2.8 in. (70mm) rotisserie
	Drip tray
	Four extra-long bottles
	1 pack mesh
	Extra-long bottle gripper
	Orbital platform
	Linear platform

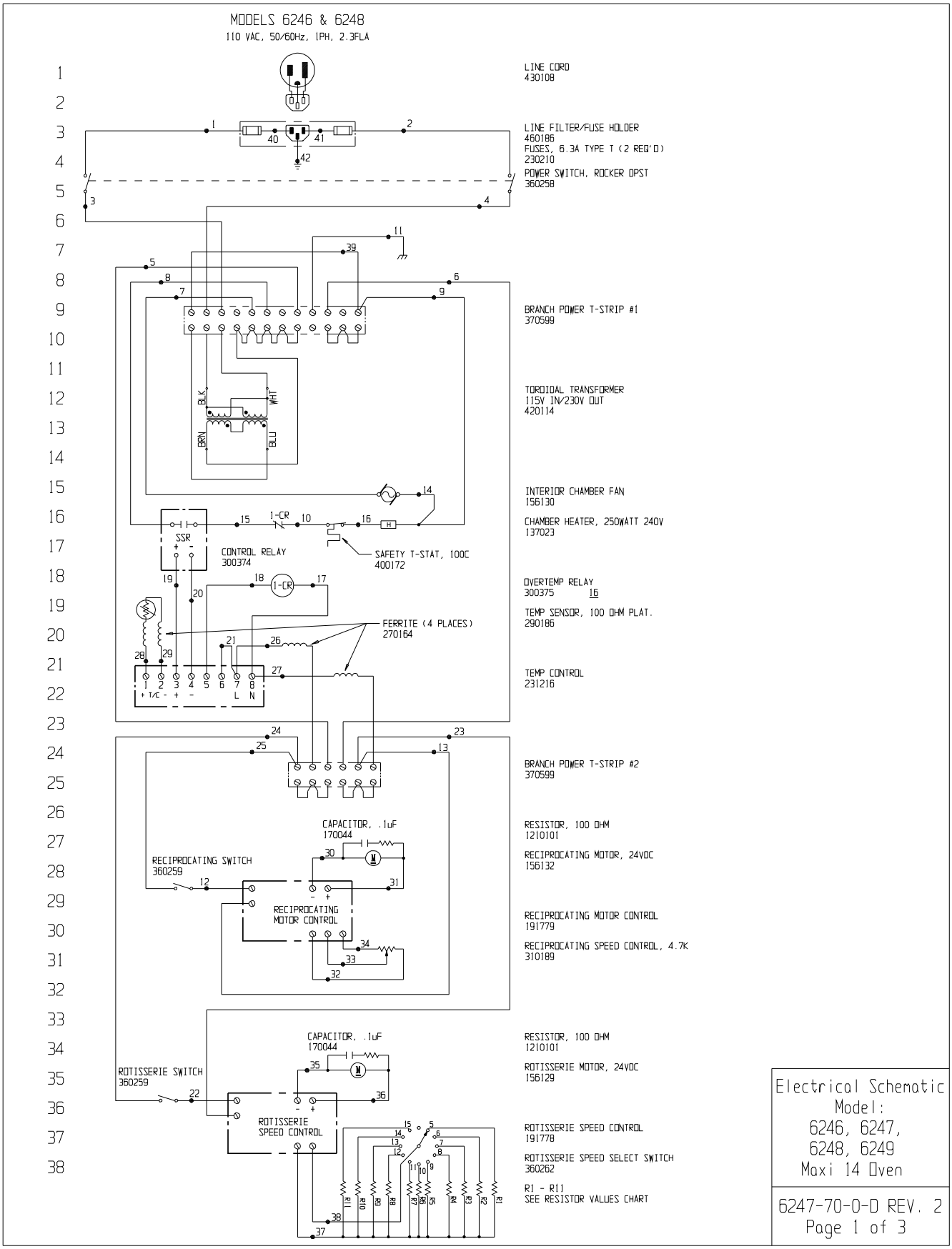
Ordering Information

110V	Model 6246
220V	Model 6247
Includes a 1.4 in. (35mm) stainless steel rotisserie, drip tray, 4 medium bottles, 1 pack mesh, 1 large bottle gripper, orbital platform and linear platform.	
110V	Model 6248
220V	Model 6249
Includes a 2.8 in. (70mm) stainless steel rotisserie, drip tray, 4 extra-long bottles, 1 pack mesh, 1 extra-long bottle gripper, orbital platform and linear platform.	
Accessory Pack	**Part Number**
Includes 2 medium bottles and a bottle gripper	222060
Additional Accessories	
Rotisseries	
Stainless Steel Rotisserie for 1.4 in. (35mm) bottles (bayonet fitting)	222034
Stainless Steel Rotisserie for 2.8 in. (70mm) bottles (bayonet fitting)	222035
Delrin Plastic Rotisseries for Disposable Tubes	222042
1 x 1.7 fl. oz. (30 x 50ml) tubes and 0.8 x 0.5 fl. oz. (25 x 15ml) tubes	
Delrin Plastic Rotisseries for Disposable Tubes	222043
0.54 x 1.7 fl. oz. (16 x 50ml) tubes and 1.7 x 0.5 fl. oz. (50 x 15ml) tubes	
Delrin Plastic Rotisseries for Disposable Tubes	222044
1.5 x 1.7 fl. oz. (44 x 50ml) tubes	

Additional Accessories (continued)	
Hybridisation Bottles	
Extra Long Bottle, 2.8 x 11.8 in. (70 x 300mm)	110094
Long Bottle, 1.4 x 11.8 in. (35 x 300mm)	110095
Medium Bottle, 1.4 x 9.8 in. (35 x 250mm)	110096
Short bottle, 1.4 x 5.9 in. (35 x 150mm)	110097
Bottle Gripper: 11.8 in. (300mm)for extra long bottles	222055
Bottle Gripper: 11.8 in. (300mm) for long, medium bottles	2200056
Bottle Gripper: 5.9 in. (150mm) for short bottles	2200057
Nylon Mesh	
5 Large mesh sheets, 9 x 9 in. (23 x 23cm)	222053
5 Small mesh sheets, 4 x 6 in. (10 x 15cm)	222052

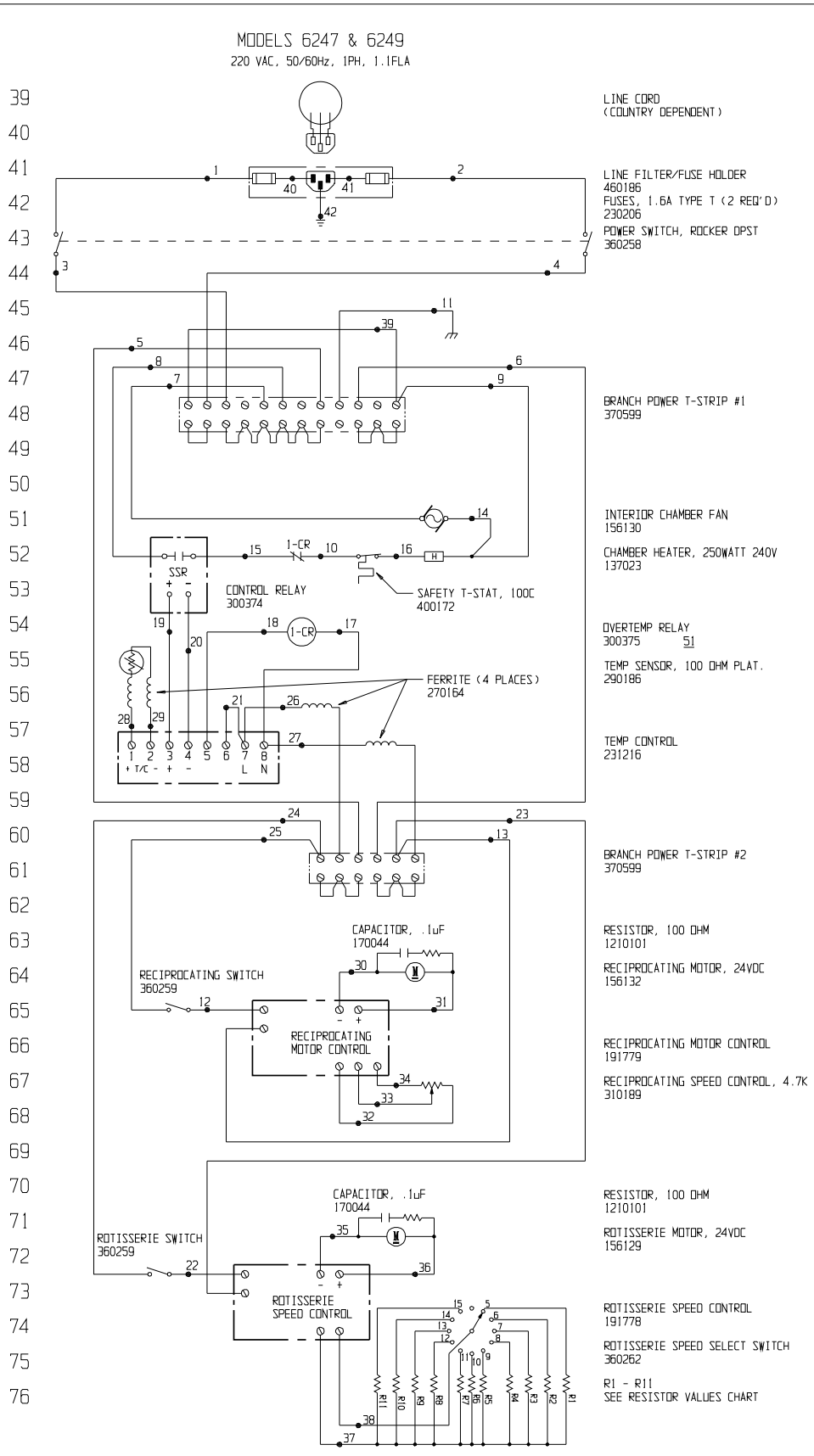
Conforms to IEC1010 Pollution Degree 2.

Conforms to Electrical Safety Standards described in IEC1010.



Electrical Schematic
Model:
6246, 6247,
6248, 6249
Maxi 14 Oven

6247-70-0-D REV. 2
Page 1 of 3



Electrical Schematic
Model:
6246, 6247,
6248, 6249
Maxi 14 Oven

6247-70-0-D REV. 2
Page 2 of 3

WIRE REFERENCE CHART					
NO.	SIZE	COLOR	NO.	SIZE	COLOR
1	.75MM²	BROWN	26	.75MM²	BROWN
2	.75MM²	BLUE	27	.75MM²	BLUE
3	.75MM²	BROWN	28	SENSOR	RED
4	.75MM²	BLUE	29	SENSOR	WHITE
5	.75MM²	BROWN	30	.5MM²	RED
6	.75MM²	BLUE	31	.5MM²	BLACK
7	.75MM²	BROWN	32	.5MM²	PINK
8	.75MM²	BROWN	33	.5MM²	GREY
9	.5MM²	BLUE	34	.5MM²	RED
10	.5MM²	BROWN	35	.5MM²	RED
11	.75MM²	GRN/YEL	36	.5MM²	BLACK
12	.75MM²	BROWN	37	.5MM²	PINK
13	.75MM²	BLUE	38	.5MM²	PINK
14	.75MM²	BLUE	39	.75MM²	BLUE
15	.5MM²	BROWN	40	.75MM²	BROWN
16	.5MM²	BROWN	41	.75MM²	BLUE
17	.75MM²	BLUE	42	.75MM²	GRN/YEL
18	.75MM²	BROWN			
19	.22MM²	RED			
20	.22MM²	BLUE			
21	.75MM²	BROWN			
22	.75MM²	BROWN			
23	.75MM²	BLUE			
24	.75MM²	BROWN			
25	.75MM²	BROWN			

ROTISSERIE SELECT RESISTOR VALUES CHART		
SYM	OHMS	STK. NUMBER
R1	511	1205110
R2	680	1210681
R3	820	1210821
R4	1K	1210102
R5	1.2K	1210122
R6	1.3K	1201301
R7	1.6K	1201601
R8	1.8K	1201801
R9	2K	1202001
R10	2.2K	1202201
R11	2.4K	1202401

REV	ECN NO.	DATE	BY	CAD	APPD	DESCRIPTION OF REVISION
2	DV-292	05-02-08	JAS	JAS	CCS	6247/6249 COUNTRY DEPENDENT CORDSET
1	N/A	12-10-03	JAS	JAS	LDN	ADD MODELS 6248 & 6249
0	N/A	9-16-03	GM	GM	MSB	RELEASED FOR PRODUCTION

ATTENTION
OBSERVE PRECAUTIONS
ELECTROSTATIC
SENSITIVE DEVICES

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BOX 649, MARIETTA, OHIO 45750

MODEL/PART NAME: 6246, 6247, 6248 & 6249 MAXI 14 OVENS
DWG TITLE: ELECTRICAL SCHEMATIC
DWN: GM | CAD: GM | APPD: MSB | DATE: 7-23-03 | SCALE: NONE
MATERIAL:
PAINT COLOR:
TOLERANCE UNLESS OTHERWISE SPECIFIED
ANGLES: DECIMAL: .XX=± .XXX=±
DRAWING NUMBER 6247-70-0
SIZE D

Electrical Schematic
Model:
6246, 6247,
6248, 6249
Maxi 14 Oven

6247-70-0-D REV. 2
Page 3 of 3

Troubleshooting Guide for Nucleic Acid Hybridisations

Our bottle system is intrinsically simpler and safer to use than other methods of hybridisation such as hybridisation in bags or plastic boxes. In the Maxi 14 Hybridisation Oven, the temperature of the solutions is precisely controlled and regulated, and in addition, the rotisserie device ensures that the solutions are continuously and evenly distributed over the membrane. Thus, the optimum conditions for hybridisation and washing are maintained throughout when using this system. However, during the transfer of protocols from bags to bottles, some minor adjustments to the protocols may be necessary.

When loading the mesh and membranes into the bottles, air bubbles should be avoided. Ensure that the Oven is positioned on a flat surface so that the probe solution is distributed evenly along the length of the bottles and that there is sufficient probe solution to cover the entire membrane. On occasions, the mesh and membrane can become tightly rolled up in the bottle. This occurs if the mesh is loaded incorrectly (see Figure 3-1 in Section 3).

Background Reduction - General

All solutions for nucleic acid hybridisations should be prepared using distilled water and highest quality reagents in clean glassware. In particular, water with a high organic content will cause bad background problems. Formamide should be freshly de-ionised. Membranes should always be handled wearing gloves or with forceps. The following should also be considered:

Pre-Hybridisation Procedure

Pre-hybridisation is required to block the sites on the nylon membrane, which the probe would otherwise bind to non-specifically. Failure to carry out adequate pre-hybridisation results in high backgrounds. If dextran sulphate is used in the hybridisation solution, then it must also be included in the pre-hybridisation solution.

Washing Procedure

Stringency washing should be carried out as follows, using large volumes (approximately 3.4 fl. oz. [100ml]) of the following solutions which should be pre-warmed to the required temperature:

1. 2 x 15 minutes with 2 x SSPE (SSC), 0.1% SDS at 65°C
2. 1 x 30 minutes with 2 x SSPE (SSC), 0.1% SDS at 65°C
3. 1 x 10 minutes with 0.1 x SSPE (SSC), 0.1% SDS at 65°C

All wash solutions should be pre-warmed to the appropriate temperature. An initial room temperature wash is not recommended and this can cause background problems.

The final wash is a high stringency wash. Use of a hand held monitor to give an indication of the decrease in radioactivity as the washes progress is recommended and should give some indication as to whether this final wash should be carried out.

In general terms, the stringency of hybridisation and washing steps is increased by increasing the temperature, or by decreasing the salt concentration. Hybridisation should be carried out under relatively low stringency conditions compared to the washing procedures. It is generally simpler and more effective to adjust the stringency during the washing steps by altering the salt concentration rather than the temperature.

Probe Preparation

The final probe concentration should be in the region of 25-50ng/ml of hybridisation solution, at approximately 1-5 x 106cpm/ml (counts per minute).

The optimum length of probe is approximately 500-800bp. Purification of the labelled probe to remove unreacted triphosphates will reduce background problems, and is recommended for all hybridisations. Probe solutions should be pre-warmed to the hybridisation temperature and care should be taken to ensure the membrane is not exposed to the concentrated probe solutions if adding it directly to the bottles.

Summary of Factors Resulting in High Backgrounds

- Hybridisation solutions and/or wash solutions not pre-warmed before use.
- Probe concentrations too high or probe not denatured. When transferring hybridisation protocols to bottles the volumes will be reduced. Ensure that probe concentrations are adjusted accordingly.
- Unincorporated nucleotides not removed from probe solution.
- Insufficient pre-hybridisation or blocking agents in pre-hybridisation and hybridisation solutions (e.g. Denhardt's reagent and salmon sperm DNA). An adequate pre-hybridisation is important to block non-specific hybridisation to the membrane.
- Hybridisation and/or washing conditions not stringent enough:

  (i) Decrease salt concentration.

  (ii) Increase temperature.

  (iii) Increase concentration of SDS.

  (iv) Increase wash times.
- Membranes drying out. This may often be the cause of an apparent overlap problem and may result from:

  (i) Too low a probe volume.

  (ii) Too slow a change over of solutions, particularly when bulk processing.

  (iii) Oven not level.

  (iv) Excessive variable axis angle.
- Residual agarose on membranes may cause foggy backgrounds. Membranes should be rinsed in 2 x SSC to remove residual agarose and excess salt after blotting and prior to fixing (especially following vacuum blotting).
- Multiple filters not separated by mesh in bottles.
- Autoradiography problems. Random black spots and "lightening flash" markings on autoradiographs may be due to static electricity.

Summary of Factors Resulting in Lower Signal Than Expected

Summary of Factors Resulting in a Signal Lower than Expected

1. Insufficient exposure time of membrane to film during autoradiography.

2. Inefficient transfer and/or binding of nucleic acids to the nylon membrane.

3. Target sequence present at very low copy numbers. Increase the amount of sample loaded on to the gel.

4. Probe sequence not present in sufficient quantities. Increase the concentration of probe or include 10% dextran sulphate, which reduces the solvent volume and has the same effect.

5. No probe homology.

6. Double stranded DNA probe was not denatured - see standard protocols. Alternatively, probe degraded. This is more likely to occur when using RNA probes.

7. The specific activity of the probe was too low. Consider factors such as the probe concentration during the labelling reaction, half-life of radiolabelled triphosphates, etc.

8. Hybridisation and/or washing conditions were too stringent:

   (i) Increase salt concentration.

   (ii) Decrease temperature.

   (iii) Reduce concentration of SDS.

   (iv) Reduce wash times.

9. The hybridisation time was too short.

Hybridisation Bottle Safety

Use of the Bottle Gripper

Part No 222057 - for 6 in. (15cm) short bottles

Part No 222056 - for 11.8 in. (30cm) medium or long bottles

Part No 222055 - for 11.8 in. (30cm) extra long bottles

This silicone rubber safety sleeve fits neatly over all our standard bottles and is included with every Oven we ship.

- Helping you to safely tighten and unscrew caps.
- Making handling of hot bottles easier.

For more details and ordering information, contact the Technical Services Department or your local Thermo Distributor.

Bottle Care

Our Hybridisation Bottles are made of thick walled borosilicate glass, which protects users from radiation and has excellent long-term reliability.

- It is important to check your bottles regularly for chips, stress fractures and cracks. If any of these are noted, discard the bottle.
- Ensure bottles are stored either in a suitable rack or with caps replaced between experiments. This will protect the bottle and sealing area.
- Replace O-rings when worn or leaky. Replace all O-rings every six months.
- Wear protective gloves to protect your hands in the event of accidental breakage.
- Never over-tighten caps on bottles. Hand-tight is sufficient.

Caution Dropping or over-tightening lids can cause cracking and failure. Lids should be inspected prior to each use. ▲

Caution If the bottle cap is difficult to unscrew, NEVER ATTEMPT to force the bottle cap open. Allow the bottle to cool and retry. If the cap remains stuck, discard the bottle. ▲

Caution The bottles should not be used at temperatures above 70°C. ▲

Declaration of Conformity

Manufacturer's Name:	Thermo Fisher Scientific
Manufacturer's Address:	401 Millcreek Road Marietta, Ohio 45750 U.S.A.
Product Description:	Maxi 14 Hybridization Ovens
Product Designations:	**6246 through 6249**
Year of Initial Marking (CE):	2005
Affected Serial Numbers:	**Release 1** (Release Level [REL#] shown on Serial Tag)

These products conform to the following European Union Directive(s):

EMC:	89/336/EEC
LVD:	73/23/EEC

These products conform to the following Harmonized, International and National Standards:

EMC:	**LVD:**
EN 50081-1:1992 EN 50082-1:1992	EN 60335-1:1994

Richard L. Miller, CQE
Regulatory Compliance Manager

ThermoFisher
SCIENTIFIC

27 February 2007

Rev. 1

THERMO FISHER SCIENTIFIC STANDARD PRODUCT WARRANTY

The Warranty Period starts two weeks from the date your equipment is shipped from our facility. This allows for shipping time so the warranty will go into effect at approximately the same time your equipment is delivered. The warranty protection extends to any subsequent owner during the first year warranty period.

During the first year, component parts proven to be non-conforming in materials or workmanship will be repaired or replaced at Thermo's expense, labor included. Installation and calibration are not covered by this warranty agreement. The Technical Services Department must be contacted for warranty determination and direction prior to performance of any repairs. Expendable items, glass, filters and gaskets are excluded from this warranty.

Replacement or repair of components parts or equipment under this warranty shall not extend the warranty to either the equipment or to the component part beyond the original warranty period. The Technical Services Department must give prior approval for return of any components or equipment. At Thermo's option, all non-conforming parts must be returned to Thermo Electron Corporation postage paid and replacement parts are shipped FOB destination.

THIS WARRANTY IS EXCLUSIVE AND IN LIEU OF ALL OTHER WARRANTIES, WHETHER WRITTEN, ORAL OR IMPLIED. NO WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE SHALL APPLY. Thermo shall not be liable for any indirect or consequential damages including, without limitation, damages relating to lost profits or loss of products.

Your local Thermo Sales Office is ready to help with comprehensive site preparation information before your equipment arrives. Printed instruction manuals carefully detail equipment installation, operation and preventive maintenance.

If equipment service is required, please call your Technical Services Department at 1-888-213-1790 (USA and Canada) or 1-740-373-4763. We're ready to answer your questions on equipment warranty, operation, maintenance, service and special application. Outside the USA, contact your local distributor for warranty information.

Rev. 3 2/07

ISO 9001 REGISTERED

THERMO FISHER SCIENTIFIC INTERNATIONAL DEALER WARRANTY

The Warranty Period starts two months from the date your equipment is shipped from our facility. This allows for shipping time so the warranty will go into effect at approximately the same time your equipment is delivered. The warranty protection extends to any subsequent owner during the first year warranty period. Dealers who stock our equipment are allowed an additional six months for delivery and installation, provided the warranty card is completed and returned to the Technical Services Department.

During the first year, component parts proven to be non-conforming in materials or workmanship will be repaired or replaced at Thermo's expense, labor excluded. Installation and calibration are not covered by this warranty agreement. The Technical Services Department must be contacted for warranty determination and direction prior to performance of any repairs. Expendable items, glass, filters, reagents, tubing, and gaskets are excluded from this warranty.

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THIS WARRANTY IS EXCLUSIVE AND IN LIEU OF ALL OTHER WARRANTIES, WHETHER WRITTEN, ORAL OR IMPLIED. NO WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE SHALL APPLY. Thermo shall not be liable for any indirect or consequential damages including, without limitation, damages relating to lost profits or loss of products.

Your local Thermo Sales Office is ready to help with comprehensive site preparation information before your equipment arrives. Printed instruction manuals carefully detail equipment installation, operation and preventive maintenance.

Contact your local distributor for warranty information. We’re ready to answer your questions on equipment warranty, operation, maintenance, service and special application.

Rev. 4 2/09

ISO 9001 REGISTERED

HYBRIDISATION GUIDE

USER INSTRUCTION MANUAL

Manual 7222060 Rev. 0

HYBRIDISATION GUIDE
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HYBRIDISATION BOTTLE SAFETY: It is important to follow correct safety procedures when using Hybridisation Bottles. Please see Appendix II for details

CHAPTER 1
HYBRIDISATION GUIDE
INTRODUCTION

The immobilisation of nucleic acids on a solid support and subsequent detection of specific sequences of interest is one of the fundamental techniques of modern molecular biology. A number of different techniques may be used to analyse target sequences from various sources. These techniques are all variations on a theme, which can be summarised according to the source of DNA or RNA as follows: -

1. **Dot/Slot Blot Hybridisation**
   A sample of unfractionated DNA or RNA immobilised on a membrane.
2. **Colony/Plaque Blot Hybridisation**
   DNA derived from cultured bacteria directly or from bacteriophage plaques and immobilised on a membrane.
3. **Southern/Northern Blot Hybridisation**
   DNA or RNA, which has been size fractionated by gel electrophoresis and then transferred to a membrane.
4. **Western Blot Hybridisation**
   Western blotting refers to the detection of polypeptides blotted on to nitrocellulose with antibodies, and is outside the scope of this manual.

In each case, the basic principle remains the same. The nucleic acid for analysis is immobilised on a solid support and then hybridised with a specific, single-stranded, Hybridisation 'probe' complementary in base sequence to the region of interest, and labelled to facilitate its detection.

The solid support most widely used for Hybridisation reactions is the nylon Hybridisation membrane. This offers several advantages over alternative matrices such as nitrocellulose, most notably high physical strength and binding capacity. The DNA is immobilised on the support by baking or UV cross-linking.

The Thermo range of Hybridisation equipment offers a complete system for the safest and most efficient processing of all types of blot. The equipment is invaluable for every stage of the nucleic acid immobilisation and detection procedure, from the initial electrophoretic separation, through to vacuum transfer and finally, Hybridisation and washing. The procedures outlined in this booklet are intended as general guidelines for the use of Thermo Hybridisation Ovens and may not be ideally suited for every research application.

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For further information, the user is recommended to refer to the following laboratory manuals (in addition to literature cited in the references): -

Fritsch, J. & Maniatis, T. (1989)
Molecular Cloning: A Laboratory Manual
2nd Edition Sambrook, Cold Spring Harbour Laboratory Press

Edited by: Hames, B.D. & Higgins, S.J. (1985)
Nucleic Acid Hybridisation: A Practical Approach
IRL Press, Oxford

Dot/Slot Blotting

1. DNA samples, prepared from cell lysate, must first be denatured to form single-stranded DNA. Heat to 100°C and incubate for 5 minutes.
2. Chill DNA samples on ice for 5 minutes.
3. Cut two pieces of Whatman 3MM™ filter paper and one sheet of membrane to the size of the Dot blotting unit. Pre-wet the membrane and filter paper in 6 x SSPE (SSC), then place in the unit and secure the lid. If samples are to be spotted on manually, pre-wet the membrane in 6 x SSPE (SSC) and place on dry filter paper.
4. Pipette samples into the wells for binding of the DNA to the membrane and follow manufacturer's instructions to operate apparatus correctly.

   If samples are spotted manually, apply 0.5-1.0µl aliquots and allow to dry between applications to prevent excessive spreading.
5. Dismantle the apparatus and remove the membrane.
6. Place the membrane on a sheet of Whatman 3MM™ filter paper saturated with **denaturing solution** for 1 minute.
7. Transfer the membrane to a sheet of filter paper saturated with **neutralising solution** for one minute.
8. Place the membrane on dry filter paper and allow to air dry.
9. Fix the DNA by baking the membrane for 1 hour at 80°C, or by UV cross-linking. UV cross-linking is the most efficient method of fixing the DNA to Hybridisation membranes and is particularly useful if reprobing is to be carried out.
10. After fixing the DNA the membrane is ready for Hybridisation. Membranes not used immediately may be stored between sheets of Whatman 3MM™ in sealed plastic bags at 4°C.

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Colony/Plaque Blotting

Colony Blotting

1. Grow bacteria on agar containing the appropriate selective antibiotic until colonies are approximately 0.5-1mm in diameter.
2. Place a Hybridisation membrane cut to the size of the plate on to the surface of the agar. Orientation points should be marked at this stage by piercing the membrane at three asymmetric points with a sterile needle.
3. Replica filters may be prepared from the initial colony lift at this stage by pressing a second Hybridisation membrane firmly on to the template membrane for a few seconds. The simplest way to do this is by sandwiching the membranes between glass plates lined with a few sheets of Whatman 3MM™ paper, and pressing them firmly together. Carefully separate the membranes to avoid smearing the colonies. This replica, and any subsequent replicas, should also be marked with orientation points.
4. The replica membranes are then placed colony side up on to fresh agar plates containing the appropriate selective antibiotic and incubated at 37°C until colonies of 0.5-1mm are present on the surface of the membrane. At this stage, it is possible to amplify low copy number plasmids (relative to cellular DNA) by transferring the filters on to agar plates containing chloramphenicol at 200μg/ml and incubating for 10 hours at 37°C.
5. Place the Hybridisation membranes on Whatman 3MM™ paper saturated in **neutralising solution** for 3 minutes then repeat with fresh **neutralising solution**.
6. Rinse the membranes briefly in 2 x SSPE (SSC), then air-dry on a sheet of filter paper.
7. Fix the DNA to the membranes either by baking for two hours at 80°C, or by UV crosslinking.
8. After fixing the DNA, membranes may be stored between sheets of Whatman 3MM™ paper in sealed plastic bags at 4°C.
9. Prior to Hybridisation, it may be advantageous to carry out a pre-washing step to remove colony debris and hence reduce background noise.

Pre-washing is carried out as required by incubating in at least 200ml of **prewash solution** (see Appendix I) for at least 30 minutes at 50°C. Longer incubations and several changes of buffer will assist in pre-washing. When the colonies are sufficiently broken up, residual colony debris may be removed by gently rubbing the colonies with a gloved finger.

This procedure is particularly advantageous when screening with oligonucleotides.

Plaque Blotting

The above procedure, with some minor modifications may also be utilised for the screening of bacteriophage plaques as follows: -

1. Aliquots of the bacteriophage stock should be mixed with plating cells and plated in soft agarose. Incubate at 37°C until plaques are approximately 0.2-0.5mm in diameter (approx. 10-12 hours).
2. Chill the plates for about 1 hour to set the top agarose.
3. Place a Hybridisation membrane cut to size (or pre-cut disc) on to the surface of the agarose and leave for at least 30 seconds. Orientation points should be marked with a sterile needle. Further replicas may be prepared by leaving the Hybridisation membrane for progressively longer periods of time on the surface of the agarose.
4. To process membranes further; proceed as from stage number 5 as for ‘Colony Blotting’. Pre-washing the filters (stage 9) should not be necessary.

Southern Blotting

1. Size fractionation of the DNA is carried out by agarose gel electrophoresis (a suitable range of horizontal gel apparatus is available from Thermo Scientific). Before transfer to Hybridisation membranes, the DNA in the agarose must be treated to ensure efficient transfer and to generate single-stranded DNA suitable for Hybridisation. Gentle agitation of the gel is essential to prevent damage to the gel during these steps. The shakers in Shake ‘n’ Stack, Midi Dual 14 and Maxi 14 are ideal for this purpose.
2. Depurination to break the DNA into smaller fragments suitable for transfer is recommended for transfer of all DNA fragments large than 10kb and may assist transfer of smaller fragments. Place the gel in a solution of 0.25M HCl for 10 minutes at room temperature with gentle shaking.

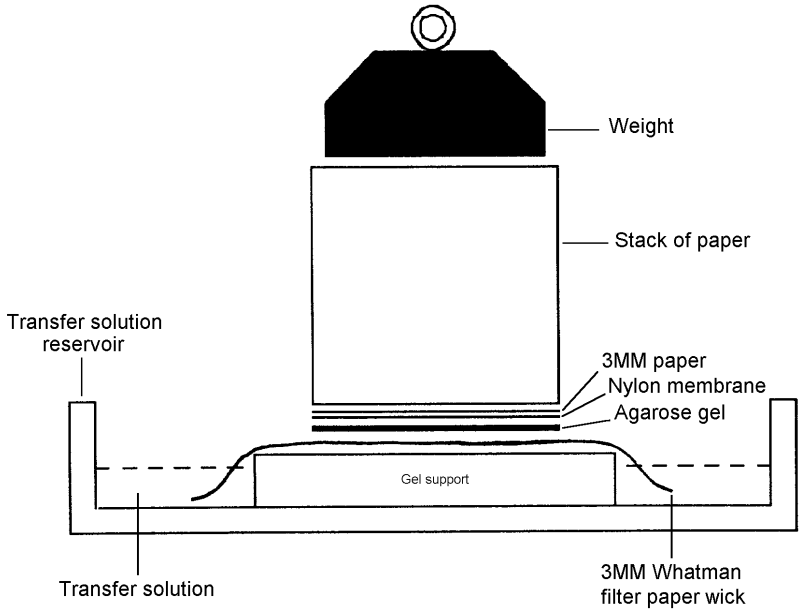
3. Replace the **depurinating solution** with **denaturing solution**. Denaturation for at least 30 minutes is recommended for most applications with a change of solution after 15 minutes.
4. Replace **denaturing solution** with **neutralising solution** and gently shake the gel for least 30 minutes, replacing with fresh solution after 15 minutes.
5. The DNA is then transferred to a Hybridisation membrane cut to the size of the gel. At this stage, a corner of the membrane should be cut to assist later in orientation. Transfer may be carried out by capillary blotting, or more efficiently by vacuum blotting. Vacuum blotting offers significant advantages over capillary blotting, namely: -
    a) Transfer is completed in 1 hour, instead of at least 4 hours for capillary blotting, or more usually overnight.
    b) Superior results are obtained with vacuum blotting. Because of the rapid transfer time, there is less lateral diffusion of the DNA during transfer to the Hybridisation membrane. This results in sharper bands on autoradiographs. Excellent blots may be prepared, quickly and conveniently, using the Thermo Vacu-Aid apparatus.

Capillary Blotting

1. Capillary blotting requires a buffer reservoir containing 10-20 x SSPE (SSC) (e.g. a bioassay dish) with a raised support for the gel (e.g. an inverted sandwich box). Two sheets of Whatman 3MM™ filter paper cut to the same width and slightly longer than the gel should be pre-wet in 10-20 SSPE (SSC) and placed across the raised support so that the ends are submerged in the buffer reservoir. This serves as a wick for the flow of the buffer through the gel (see *Figure 2.2: Capillary Blot Assembly*).
2. Invert the gel and place centrally on the filter paper. Any filter paper exposed at the edge of the gel should be covered in Saran Wrap™, used X-ray film or similar, to prevent flow of buffer around the edge of the gel.

3. Place a sheet of Hybridisation membrane cut to the size of the gel and marked for orientation on to the surface of the gel, taking care to avoid air bubbles. The membrane should only be handled wearing gloves or using forceps. Then cover the membrane with two sheets of Whatman 3MM™ paper cut to just larger than the gel.
4. Place a stack of absorbent paper towels on top of the filter paper, weighted down with a 1kg weight. The stack of towels should be approximately 5cm deep, sufficient to continue drawing buffer through the gel until transfer is complete, which takes at least 4 hours. The stack of towels should also be level to ensure even transfer. Take care to ensure that the stack of towels is not in contact with the buffer wick, which would cause a short circuit of buffer, bypassing the gel. Usually it is convenient to transfer overnight.
5. After blotting is complete, the apparatus is dismantled. At this stage it is possible to mark the position of the wells on the Hybridisation membrane with a soft pencil. The membrane is then rinsed briefly in 2 x SSPE (SSC) and air-dried on a sheet of dry filter paper.
6. Fix the DNA to the Hybridisation membrane, either by baking at 80°C for 2 hours, or by UV cross-linking.
7. After fixing the DNA, the membrane is ready for Hybridisation. Membranes not hybridised immediately may be stored between sheets of Whatman 3MM™ paper in sealed plastic bags at 4°C.

***Figure 2.1**: Capillary Blot Assembly*



Northern Blotting

Electrophoresis of RNA should be carried out in a denaturing gel system. Maniatis *et al* 1989 gives details of running denaturing RNA gels using formaldehyde or glyoxal.

1. After electrophoresis of the RNA in a denaturing gel the transfer can be set up as described for DNA gels (see Southern Blotting above). The denaturating step 3 and the neutralising step 4 of the gel are not required, as the RNA is denatured in the gel. However, some researchers recommend reduced stringency denaturation and neutralisation steps to facilitate transfer of large RNA molecules as follows: -

50mM NaOH, 10mM NaCl	30 minutes
100mM Tris HCl Ph7.5	30 minutes

   Gentle agitation of the gel is essential to prevent damage to the gel during these steps. The shaker in the Shake 'n' Stack, Midi Dual 14 and Maxi 14 are ideal for this purpose.

2. RNA transfer is carried out in 10-20 x SSPE, using the same procedure as outlined for DNA gels in Southern Blotting section above.

3. After transfer of the RNA to the Hybridisation membrane is completed, fixing of the RNA is carried out by baking at 80°C for 2 hours, or by UV cross-linking.

4. After fixing the RNA, the membrane is ready for Hybridisation. Membranes not used immediately may be stored between sheets of Whatman 3MM™ paper in sealed plastic bags at 4°C.

CHAPTER 3
HYBRIDISATION GUIDE
HYBRIDISATION PROCEDURES

Nucleic acid hybridisations are conveniently performed in the dedicated Hybridisation equipment available from Thermo.

The Hybridisation Oven range consists of incubators with integral rotisserie devices, which can accommodate 4, 10 or 14 Hybridisation bottles, 35mm in diameter, in order to perform Hybridisations in minimal probe volumes with fluid moving continually over the membrane.

Southern Blot DNA Hybridisations

The following protocol is broadly applicable to Hybridisations using DNA probes of 50bp and above, following transfer and fixing of DNA to membranes as described in Chapter 2. For Hybridisations using oligonucleotides refer to Chapter 6. The Hybridisation procedure consists of four stages: -

1. Prehybridisation
2. Hybridisation
3. Stringency washing
4. Autoradiography

For detailed notes on Hybridisation specific to Thermo equipment, refer to 'Notes for Nucleic Acid Hybridisation' on page 11.

1. **Prehybridisation** is carried out by incubating the membrane in **Southern Blot Prehybridisation Buffer** (for recipe see page 30). Denature salmon sperm DNA by boiling for 5 minutes and then chilling on ice. Add the denatured salmon sperm DNA to the buffer to a final concentration of 50μg/ml.
2. Incubate with agitation or in a rotisserie for a least 1-hour at 65°C.
3. The volume of prehybridisation buffer required varies according to the Hybridisation system being utilised. In general terms, the minimum volume of buffer should be used such that the membrane is covered by the fluid at all times (approximately 0.1ml/cm^2), or if in Hybridisation bottles, 10ml for a large bottle and 5ml for a small bottle.

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4. Denature the purified labelled probe by heating to 100°C and incubating for 5 minutes, then chill on ice and either add to the prehybridisation solution taking care not to pipette directly on to the membrane, or dilute the probe in pre-washed Hybridisation solution and use this to replace the prehybridisation solution.

5. **Hybridise** with agitation, or by rotating in bottles, for approximately 12 hours at 65°C.

6. Stringency washing steps are carried out as follows, using large volumes (at least 50ml) of the following solutions, which should be pre-warmed to the required temperature: -

2 x 15 minutes	with 2 x SSPE (SSC)	0.1% SDS at 65°C
1 x 30 minutes	with 1 x SSPE (SSC)	0.1% SDS at 65°C
1 x 10 minutes	with 0.1 x SSPE (SSC)	0.1% SDS at 65°C

The final wash is a high stringency wash. Use of a hand held monitor to give an indication of the decrease in radioactivity as the washes progress is recommended and should determine whether this final wash should be carried out. (See Appendix II for further notes.)

7. Wrap the membrane in Saran Wrap™ and autoradiograph at -70°C in a cassette with an intensifying screen or use a phosphoimager. Expose initially for approximately 12 hours (or overnight).

8. If the membrane is to be reprobed, at no stage should the membrane be allowed to dry out? Removal of the probe on the membrane may be carried out by boiling the membrane in 0.1 x SSPE (SSC), 0.1% SDS for 5 minutes. Check for residual probe activity by autoradiography before hybridising again.

Northern Blot RNA Hybridisation

1. Prehybridisation is carried out by incubating the membrane in **Northern Blot Prehybridisation Buffer** (recipe on page 33). Denature salmon sperm DNA by boiling for 5 minutes and then chilling on ice. Add the denatured salmon sperm DNA to the buffer, to a final concentration of 100μg/ml.

2. Incubate with agitation or in a rotisserie for at least one hour at 42°C.

3. The volume of prehybridisation buffer required varies according to he Hybridisation system being utilised. In general terms, the minimum volume of buffer should be used such that the membrane is covered by the fluid at all times (approximately 0.1ml/cm^2), or if in a Hybridisation bottle, 10-20ml for a large bottle and 5-10ml for a small bottle.

4. Denature the labelled probe by heating to 100°C and incubating for 5 minutes. Chill on ice and add to the prehybridisation solution. Depending upon the system utilised, the probe may be added directly or, alternatively, some prehybridisation buffer is removed, the probe added to this, and then the solution replaced in the Hybridisation vessel. Some researchers may prefer to use fresh Hybridisation solution.

5. Hybridise with agitation, or rotating in bottles, for approximately 12 hours at 42°C.

6. Stringency washing steps are carried out as follows using large volumes (at least 50ml) of the following solutions which should be pre-warmed to the required temperature: -

2 x 15 minutes	with 2 x SSPE	0.1% SDS at 42°C
1 x 30 minutes	with 1 x SSPE	0.1% SDS at 42°C
1 x 15 minutes	with 0.1 x SSPE	0.1% SDS at 42°C

The final wash is a high stringency wash. Use of a hand held monitor to give an indication of the decrease in radioactivity as the washes progress is recommended and will determine if the final wash should be carried out.

7. Wrap the membrane in Saran Wrap™ and autoradiograph at -70°C in a cassette with an intensifying screen or use a phosphoimager. Expose initially for 12 hours (or overnight).

8. If the membrane is to be reprobed, at no stage should the membrane be allowed to dry out? Removal of the probe on the membrane may be carried out by washing the membrane for 1-2 hours at 65°C in 5mM Tris HCl pH8.0, 0.2mM EDTA, 0.1 x Denhardt's reagent.

Notes for Nucleic Acid Hybridisations using the Thermo Range of Equipment

The Hybridisation Oven range has been designed to provide the optimum conditions for performing all types of Hybridisation and stringency washing procedures safely and simply. Hybridisations are performed in bottles to maximise user safety and to minimise probe volumes.

Placing Membranes in a Bottle

1. Place the bottles to be used in the Oven during the warm up period (approximately 1 hour).

2. Select a piece of support mesh appropriate for the size of the membrane. The recommended Hybridisation mesh is supplied by Thermo.

3. Pre-wet the mesh and Hybridisation membrane in a suitable tray containing 2 x SSPE (SSC) (see *Figure 3.1*).

4. Ensuring that the Hybridisation membrane exactly overlays the mesh, roll both up into a tight roll.

   If more than one membrane is to be hybridised in a bottle, simply overlay further meshes and membranes as required before rolling. It is important that each membrane is separated from any other by a piece of mesh. Up to five 20 x 20mm membranes can be hybridised in a single Hybridisation bottle.

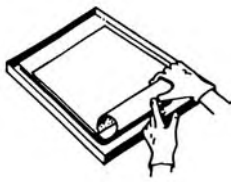
5. Place 10-15mls SSPE (SSC) into a Hybridisation Bottle and then insert the roll in such a way that the leading edge (inside the roll) and the trailing edge are positioned relative to each other as shown in Figure 3.2).

6. Place the bottle on a flat surface and then slowly unwind the membrane and mesh around the inside of the bottle by rocking and gently rolling the bottle along the surface. No air bubbles should be visible between the membrane and the bottle. If bubbles are present, the membrane should be removed and re-rolled.

   The procedure should then be repeated more gently. Rock the bottle backwards and forwards to attach the first part of the membrane to the bottle. Then roll the bottle in order to unwind the mesh and membrane.

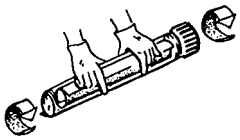
7. Continue until the membrane and mesh is fully unwound. The mesh ensures the probe will have access to all parts of the Hybridisation membrane including those parts of the membrane, which overlap.

***Figure 3: Inserting** Membranes in a Bottle*

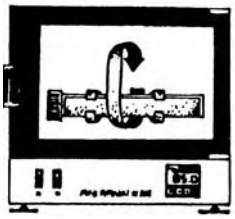


1. Shallow tray containing 2 x SSPE (SSC).
   Mesh.
   Membrane.
   Roll up mesh & membrane.

2. Place 10-15mls of SSPE (SSC) into a bottle and then insert the roll centrally.



3. Secure cap and, holding bottle horizontally, roll to catch the trailing edge of the mesh, continue rolling in the same direction until coil of mesh and membrane is well positioned.



4. Pour out SSPE (SSC) and replace with pre-warmed prehybridisation buffer.

5. Place the bottle in the Oven so that it rotates in the Oven in the same direction, as it was unrolled in step 3.

Prehybridisation

1. Once the membrane is in place in the bottle the SSPE (SSC) can be discarded and replaced with prehybridisation buffer.

   This is easily done by removing the cap, pouring off the SSPE (SSC) and then pouring in the prehybridisation buffer. All the solutions used during Hybridisation should be pre-warmed before use. 10-20ml solution is recommended for medium bottles and 5-10ml for small bottles. Higher volumes will be required if there is more than one membrane in the bottle,
   e.g. 15-25ml.

2. Replace the cap on the bottle and insert into the rotisserie.

Hybridisation

1. Denature the purified probe by boiling for 5 minutes, then store on ice. If the volume to be used for Hybridisation is substantially different to that used previously for Hybridisation in bags or boxes, ensure that the quantity of the probe is adjusted accordingly to maintain the correct probe concentration. If this is not done, high background may result.

2. Remove the bottle from the Hybridisation Oven and unscrew the cap.

3. If the same buffer is to be used for Hybridisation as for prehybridisation simply pipette the probe into the prehybridisation fluid in the bottle. Take care to avoid pipetting the probe directly on to the membrane as this will result in hot spots. Alternatively, dilute the probe in pre-warmed Hybridisation buffer outside the bottle. Pour off the prehybridisation buffer and replace with the probe solution.

4. Replace the cap and gently agitate the bottle to ensure an even distribution of the probe in the Hybridisation solution.

5. Place the bottle back in the Hybridisation Oven, switch on the rotisserie and leave it to hybridise for the required time period.

Washing

Method 1 - Washing in Hybridisation Bottles

1. Remove the bottle from the Oven.

2. Pour off the Hybridisation buffer and then **half fill** the bottle with the first pre-warmed wash solution (approx. 100ml) and replace the cap.

   NOTE: Room temperature washing or washing with solutions, which have not been pre-warmed, may result in background problems (see Chapter 7, page 27).

3. Replace the bottle in the Oven and leave for the required time period.

   If transferring from sandwich box or plastic bag methodology, the times and temperatures are usually those, which are currently being used, approximately 20 minutes per wash step.

4. Repeat steps 1-3 for each additional wash. The wash solutions, temperatures etc., should be those recommended by the membrane manufacturer, or as detailed in Chapter 7, Washing Procedure.

NOTE: All wash solutions should be pre-warmed for best results.

Method 2 - Washing in a Sandwich Box

Some scientists prefer to remove the membranes from the bottles and wash them all in one container. Washing may be performed very simply on the shaking platform in the Shake 'n' Stack, Midi Dual or Maxi 14 Ovens and is particularly useful when several membranes are being processed simultaneously.

1. Remove the Hybridisation Bottle from the Oven. Pour off the Hybridisation buffer.

2. Using a pair of forceps, carefully remove the membranes and place them in a suitable container with a lid (e.g. sandwich box or similar).

3. Using the forceps gently unroll the membranes.

4. Add a sufficient volume of the pre-warmed first wash solution to totally immerse the membranes. Replace the lid and shake on the shaking platform in the Hybridisation Oven at the required temperature for the specified time period.

NOTE: Initial room temperature washes are not recommended and may result in subsequent background problems.

5. Remove the first wash solution and replace it with an equal volume of the pre-warmed second wash solution. Replace the lid and shake at the required temperature for the required time period.

6. Step 5 should be repeated for any subsequent wash. The washing protocol using the shaking platform will be identical to that used in the bottles.

Please refer to Chapter 7 for additional information on Washing.

CHAPTER 4
HYBRIDISATION GUIDE
NUCLEIC ACID PROBES

There are now several types of nucleic acid probe available to researchers and a wide variety of radio-labelling techniques for labelling these probes.
For convenience, the types of nucleic acid probe may be classified as follows: -

1. Double-stranded DNA probes
2. Single-stranded DNA probes
3. RNA probes
4. Synthetic oligonucleotides

Double-Stranded DNA Probes

Any suitable DNA molecules (cloned or uncloned) can be used as a Hybridisation probe, for example, insert fragments from a DNA library may be excised from plasmids or bacteriophages by restriction enzyme digest and then labelled for Hybridisation. Another possibility is to use the Polymerase Chain Reaction to synthesize copies of the region of interest, which may be radio-labelled during the amplification reaction, itself, or the final product labelled post-amplification. Labelling of double-stranded DNA may be carried out by NICK TRANSLATION OR PRIMER EXTENSION. Commercially available kits enable these techniques to be carried out simply and efficiently resulting in probes of high specific activity, often requiring no further purification before adding to the Hybridisation buffer.

Removal of unincorporated nucleotides may be advantageous in reducing background. This may be simply carried out using Thermo Recovery kits.

Single-Stranded DNA Probes

Single-stranded DNA probes may be synthesized from mRNA using Reverse Transcriptase, or may be derived from fragments cloned into specialised M13 or phagemid vectors, which contain the origin of replication of a single-stranded DNA bacteriophage. Synthesis of the DNA strand complementary to the region of interest incorporating a ^{32}P labelled dNTP results in a single-stranded radio labelled probe molecule, which is then separated from the unlabelled template by gel electrophoresis (the fragment may then be extracted from the gel using Thermo Recovery kits). This technique eliminates the possibility of re-association of complementary strands, which can occur with double-stranded DNA probes.

RNA Probes

RNA probes are also derived from fragments, which have been cloned into specialised cloning vectors. These vectors contain the promoter sequences of a bacteriophage encoded DNA - dependent RNA polymerase (for example SP6 to T7) adjacent to a multiple cloning site, into which the fragment is inserted. Transcription of the cloned fragment in the presence of the specific RNA polymerase and a ^{32}P-labelled rNTP results in labelled RNA. The template DNA is removed simply by DNAse digest. Vectors of this type are available containing two phage promoters in opposite orientations adjacent to the multiple cloning site so that the strand to be transcribed may be chosen according to the phage RNA polymerase used. These RNA probes have become the probes of choice for many applications, the only limitations being the additional care required for handling RNA.

Synthetic Oligonucleotides

An oligonucleotide probe of a single defined DNA sequence may be synthesized if the target
nucleic acid sequence is available using a DNA synthesis machine or commercial service. Alternatively, pools of oligonucleotides of degenerate sequences may be synthesized, derived from an amino acid sequence of a gene product. Due to the degeneracy of the genetic code (i.e. most amino acids are specified by more than one triplet codon) a given amino acid sequence will be coded for by a number of alternative DNA sequences, one of which will represent the specific target sequence for the probe. Oligonucleotides are most commonly radio-labelled using T4 Polynucleotide Kinase to add a labelled phosphate group to the 5′ terminus.

The various protocols of radio-labelling nucleic acid probes are widely available in the published literature (e.g. Maniatis *et al*). In addition, there are several commercially available kits containing all the components required for the different types of labelling experiments.

High quality HPLC purified oligonucleotides are available online from Thermo Scientific, visit our web site at www.thermo.com/molecularbiology.

CHAPTER 5
HYBRIDISATION GUIDE
NON-RADIOACTIVE SYSTEM

The use of non-radioactive labelling techniques is becoming increasingly important for nucleic acid Hybridisation. There are currently a number of systems available. Most are based on the labelling of probes with either biotin or the hapten Digoxygenin (DIG).

Biotinylated probes are hybridised and then linked to an appropriate enzyme such as alkaline phosphatase. Visualisation is achieved by reaction of the enzyme with a chromogenic substrate.

Similarly, DIG labelled probes are detected by high affinity Fab fragments coupled to alkaline phosphatase. Visualisation is achieved using chromogenic substrates such as NBT and BCIP, which produces a dark blue colour.

Although both of these systems offer advantages over radio-labelled probes (no safety risk or stability problems), they have been recognised as less sensitive.

Recently the sensitivity of detection has been increased significantly (down to femtogram levels) by using a chemiluminescent reaction. The Böehringer DIG system with the same alkaline phosphatase conjugate will generate light with the chemiluminescent substrate AMPPD. The resultant signals produced can be detected using standard X-ray film or a phosphoimager in as little as 30 minutes. Light continues to be given off for up to 48 hours for longer developments.

The DIG System in Thermo Hybridisation Ovens

The small probe volumes, even mixing, accurate temperature control and ease of handling associated with Thermo Oven systems are equally suited to the success of non-radioactive Hybridisation.

The protocol detailed below has been developed in conjunction with the Böehringer DIG system. The prehybridisation, Hybridisation and washing stages of the protocol using the DIG labelled probe are exactly as for radio-labelled probes. The subsequent wash stages associated with detection and visualisation of the probe is often performed in sandwich boxes, but will work equally well in bottles.

This protocol has been optimised for DNA RFLP analysis. It may require minor alteration to be used for other applications.

The following protocol applies to a 20cm x 20cm membrane: -

Electrophoresis & Transfer

Electrophorese and transfer DNA fragments on to positively charged Hybridisation membrane (Böehringer Mannheim, Cat N° 1209 272). Bake at 120°C for 30 minutes to fix DNA or UV cross-link.

DNA Hybridisation

Prehybridisation and Hybridisation are carried out in bottles in a Thermo rotisserie oven. Better results have been obtained in bottles than in bags.

1. Pre-warm Hybridisation bottle containing 50ml 2 x SSC to 68°C.

2. Layer the following into a plastic box containing 50ml 2 x SSC:
   a) One piece of nylon mesh (23cm x 23cm - Thermo Scientific).
   b) The membrane, DNA side-up.
   c) Two layers of nylon mesh.
   d) One piece of 'dummy' Hybridisation membrane covering Hybridisation membrane below.
   e) One piece of nylon mesh.

   This procedure prevents high backgrounds. (Mesh and 'dummy' membrane are reusable after washing in distilled water.)

3. Roll 'sandwich' (ensuring no air bubbles are trapped) with DNA side facing inwards.
4. Place roll in Hybridisation bottle and carefully unroll 'sandwich' again ensuring no air bubbles are trapped.
5. Tip off 2 x SSC and add 20ml prehybridisation buffer pre-warmed to 68°C.
6. Prehybridise in rotisserie oven for 2 hours at 68°C.
7. Denature 300ng DIG labelled probe. Add to 15ml-prehybridisation buffer heated to 68°C.
8. Tip buffer off prehybridised membrane and add Hybridisation buffer to the bottle. Hybridise overnight in rotisserie oven at 68°C.
9. Remove Hybridisation buffer and freeze. This can be reused a further five times after heating to 95°C for 10 minutes.
10. Add 50ml 2 x SSC 0.1% SDS to bottle and roll in opposite direction to release 'sandwich'.

11. Perform washes in bottle replacing each wash with: -

2 x 5 minutes	in 50-100ml 4 x SSC	1% SDS at 68°C
2 x 15 minutes	in 50-100ml 2 x SSC	0.1% SDS at 68°C
1 x 15 minutes	in 50-100ml 0.1 x SSC	0.1% SDS at 68°C

Alternatively, remove membrane from roll and wash in plastic box: -

2 x 5 minutes	in 500ml 2 x SSC	0.1% SDS at room temp.
2 x 15 minutes	in 500ml 0.2 x SSC	0.1% SDS at 68°C

CHAPTER 6
HYBRIDISATION GUIDE
HYBRIDISATION USING OLIGONUCLEOTIDE PROBES

The Hybridisation conditions outlined in Chapter 3 are generally applicable to probes derived by cloning, or by gene amplification reactions ranging in size from 100bp to several hundred base pairs. The Hybridisation conditions of synthetic oligonucleotide probes, however, are rather more problematical.

There are two types of oligonucleotide probes: -

1. A single oligonucleotide of a defined sequence, derived from the nucleic acid sequence of the target gene.
2. A pool of oligonucleotides of degenerate sequences, which are derived from the amino acid sequence of a region of the gene product of interest. Due to the degeneracy of the genetic code (i.e. most amino acids are specified by more than one triplet codon) any particular sequence of amino acids will be specified by a number of different oligonucleotides.

   Because the Tm of synthetic oligonucleotides is much lower than for longer probes, the stringency of Hybridisation and washing procedures must be reduced and adjusted according to the base composition of the probe. An approximate value for the Tm of an oligonucleotide probe has been derived by Wallace *et al* (1979): -

   Tm (°C) = 2 x (number of AT base pairs) +
   4 x (numbers of GC base pairs).

   Hybridisations are carried out at 5°C below this temperature. For a degenerate pool of oligonucleotides, Hybridisation is carried out at 5°C below the lowest Tm for the set of sequences.

1. Prehybridise the membrane in oligonucleotide prehybridisation buffer (6 x SSPE (SSC), 10 x Denhardt's reagent, 50μg/ml denatured salmon sperm DNA) at the Hybridisation temperature for at least one hour.
2. Add the radio-labelled probe solution to the preHybridisation buffer. Alternatively, remove a small amount of preHybridisation buffer, add the probe to this and replace this solution in the Hybridisation vessel. Some researchers may prefer to use fresh Hybridisation buffer.
3. Hybridise with agitation, or by rotating in bottles, for approximately 12 hours at the Hybridisation temperature.

4. Stringency washing is carried out as follows using large volumes (at least 50ml) of the following solutions, pre-warmed to the required temperature:

2 x 15 minutes	with 6 x SSPE (SSC)	0.1% SDS at the Hybridisation temp.
1 x 2 minutes	with 6 x SSPE (SSC)	0.1% SDS at the Tm

5. Wrap the membrane in Saran Wrap™ and autoradiograph at -70°C in a cassette with an intensifying screen. Expose initially for approximately 12 hours (or overnight).

Background Hybridisation problems are much more common when using oligonucleotide probes. For this reason procedures to reduce background such as pre-washing of colony filters (Chapter 2, Colony/Plaque Blotting) and purification of the oligonucleotide to remove unincorporated nucleotides are particularly advantageous.

CHAPTER 7
HYBRIDISATION GUIDE
TROUBLESHOOTING GUIDE FOR NUCLEIC ACID HYBRIDISATIONS

Our bottle system is intrinsically simpler and safer to use than other methods of Hybridisation such as Hybridisation in bags or plastic boxes. In Hybridisation Ovens, the temperature of the solutions is precisely controlled and regulated and, in addition, the rotisserie device ensures that the solutions are continuously and evenly distributed over the membrane. Thus, the optimum conditions for Hybridisation and washing are maintained throughout when using this system. However, during the transfer of protocols from bags to bottles some minor adjustments to the protocols may be necessary.

When loading the mesh and membranes into the bottles, air bubbles should be avoided. Ensure that the Oven is sited on a flat surface so that the probe solution is distributed evenly along the length of the bottles and that there is sufficient probe solution to cover the entire membrane. On occasions the mesh and membrane can become tightly rolled up in the bottle. This occurs if the mesh is loaded incorrectly *(see Figure 3 in Chapter 3).*

Background Reduction - General

All solutions for nucleic acid Hybridisations should be prepared using distilled water and highest quality reagents in clean glassware. In particular, water with a high organic content will cause bad background problems. Formamide should be freshly de-ionised. Membranes should always be handled wearing gloves or with forceps. The following should also be taken into consideration: -

Prehybridisation Procedure

Prehybridisation is required to block the sites on the nylon membrane, which the probe would otherwise bind to non-specifically. Failure to carry out adequate preHybridisation results in high backgrounds. If dextran sulphate is used in the Hybridisation solution, then it must also be included in the prehybridisation solution.

Washing Procedure

Stringency washing should be carried out as follows, using large volumes (approximately 100ml) of the following solutions which should be pre-warmed to the required temperature: -

1. 2 x 15 minutes with 2 x SSPE (SSC), 0.1% SDS at 65°C

2. 1 x 30 minutes with 2 x SSPE (SSC), 0.1% SDS at 65°C

3. 1 x 10 minutes with 0.1 x SSPE (SSC), 0.1% SDS at 65°C

All wash solutions should be pre-warmed to the appropriate temperature. An initial room temperature wash is not recommended and can cause background problems.

The final wash is a high stringency wash. Use of a hand held monitor to give an indication of the decrease in radioactivity as the washes progress is recommended and should give some indication as to whether this final wash should be carried out.

In general terms, the stringency of Hybridisation and washing steps is increased by increasing the temperature, or by decreasing the salt concentration. Hybridisation should be carried out under relatively low stringency conditions compared to the washing procedures. It is generally simpler and more effective to adjust the stringency during the washing steps by altering the salt concentration rather than the temperature.

Probe Preparation

The final probe concentration should be in the region of 25-50ng/ml of Hybridisation solution, at approximately 1-5 x 10^6cpm/ml.

The optimum length of probe is approximately 500-800bp. Purification of the labelled probe to remove unreacted triphosphates will reduce background problems, and is recommended for all Hybridisations - Thermo Recovery kits are excellent for this purpose. Probe solutions should be pre-warmed to the Hybridisation temperature and care should be taken to ensure the membrane is not exposed to the concentrated probe solutions if adding it directly to the bottles.

Summary of Factors Resulting in High Backgrounds

1. Hybridisation solutions and/or wash solutions not pre-warmed before use.
2. Probe concentrations too high or probe not denatured. When transferring Hybridisation protocols to bottles the volumes will be reduced. Ensure that probe concentrations are adjusted accordingly.
3. Unincorporated nucleotides not removed from probe solution.
4. Insufficient prehybridisation or blocking agents in prehybridisation and Hybridisation solutions (e.g. Denhardt's reagent and salmon sperm DNA). An adequate prehybridisation is important to block non-specific Hybridisation to the membrane.
5. Hybridisation and/or washing conditions not stringent enough: -
   (i) Decrease salt concentration.
   (ii) Increase temperature.
   (iii) Increase concentration of SDS.
   (iv) Increase wash times.
6. Membranes drying out. This may often be the cause of an apparent overlap problem and may result from: -
   (i) Too low a probe volume.
   (ii) Too slow a change over of solutions, particularly when bulk processing.
   (iii) Oven not level.
   (iv) Excessive variable axis angle.
7. Residual agarose on membranes may cause foggy backgrounds. Membranes should be rinsed in 2 x SSC to remove residual agarose and excess salt after blotting and prior to fixing (especially following vacuum blotting).
8. Multiple filters not separated by mesh in bottles.
9. Autoradiography problems. Random black spots and "lightening flash" markings on autoradiographs may be due to static electricity.

Summary of Factors Resulting in a Signal Lower than Expected

1. Insufficient exposure time of membrane to film during autoradiography or phosphoimaging.

2. Inefficient transfer and/or binding of nucleic acids to the nylon membrane.

3. Target sequence present at very low copy numbers. Increase the amount of sample loaded on to the gel.

4. Probe sequence not present in sufficient quantities. Increase the concentration of probe or include 10% dextran sulphate, which reduces the solvent volume and has the same effect.

5. No probe homology.

6. Double-stranded DNA probe was not denatured - see standard protocols. Alternatively, probe degraded. This is more likely to occur when using RNA probes.

7. The specific activity of the probe was too low. Consider factors such as the probe concentration during the labelling reaction, half-life of radio-labelled triphosphates, etc.

8. Hybridisation and/or washing conditions were too stringent: -

   (i) Increase salt concentration.

   (ii) Decrease temperature.

   (iii) Reduce concentration of SDS.

   (iv) Reduce wash times.

9. The Hybridisation time was too short.

APPENDIX I
SOLUTIONS FOR NUCLEIC ACID BLOTTING & HYBRIDISATION PROCEDURES

1. 20 x SSC (1 litre)

NaCl	175g
Sodium citrate	88g

Dissolve in 800ml of H_2O and adjust pH to 7.0 with NaOH solution. Adjust the volume to 1 litre with H_2O and sterilise by autoclaving.

2. 20 x SSPE (1 litre)

NaCl	175g
NaH_2PO_4 H_2O	27.6g
Na_2EDTA	7.4g

Dissolve in 800ml of H_20 and adjust pH to 7.4 with NaOH solution. Adjust the volume to 1 litre with H_2O and sterilise by autoclaving.

3. 100 x Denhardt's Reagent

Ficoll	2g
Polyvinyl pyrollidone	2g
Bovine serum albumin (Fraction V)	2g

Dissolve in 100ml H_2O, filter and store in aliquots at -20°C.

4. Salmon Sperm DNA

Dissolve the DNA at a concentration of 1mg/ml in sterile H_2O, sonicate to a length of 200-500bp, and then stand in a boiling water bath for 10 minutes. Aliquot and store at -20°C.

5. Denaturing Solution

NaCl	1.5M
NaOH	0.5M

Sterilise by autoclaving.

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6. Neutralising Solutions

NaCl	1.5M
Tris Cl pH7.2	0.5M
Na_2EDTA	0.001M

Sterilise by autoclaving.

7. 10% Sodium Dodecyl Sulphate (SDS)

(Also known as sodium lauryl sulphate.)

8. Formamide

Formamide should be de-ionised before use. Add 5g of a mixed bed ion exchange resin (e.g. Biorad AG501) to 100ml formamide and stir for 1-2 hours. Store at -20°C.

9. Pre-wash Solution

5 x SSC
0.5% SDS
1mM Na_2EDTA

10. Prehybridisation Buffer for Southern Blots

Prepare as follows (10ml):

Solution	***Volume***	***Final Concentration***
20 x SSPE (or SSC)	3ml	6 x SSPE (SSC)
100 x Denhardt's reagent	0.5ml	5 x Denhardt's
10% SDS	0.5ml	0.5% SDS
H_2O	4.5ml	

Add denatured salmon sperm DNA to 50μg/ml.

11. Prehybridisation Buffer for Northern Blots

Prepare as follows (10ml):

Solution	***Volume***	***Final Concentration***
20 x SSPE	2.5ml	5 x SSPE
Formamide	5ml	50%
100 x Denhardt's reagent	0.5ml	5 x Denhardt's
10% SDS	0.5ml	0.5% SDS
H_2O	1.5ml	

Add denatured salmon sperm DNA to 100μg/ml.

NB: All solutions should be prepared in clean, sterile glassware using distilled water and highest quality reagents.

NB: In addition, Hybridisation membranes should always be handled wearing gloves or using forceps. Where it is necessary to sterilise the membranes, place between sheets of filter paper and autoclave for 15 minutes.

APPENDIX II
FACTORS AFFECTING STRINGENCY OF HYBRIDISATION REACTIONS

The stringency of Hybridisation determines the degree to which mismatched hybrids are permitted to form. The optimum Hybridisation temperature for DNA - DNA Hybridisations is 25°C below the melting temperature (Tm).

The Tm is affected by: -

1. The concentration of monovalent cation (M).
2. Base composition (percentage G+C content).
3. The length of the duplex in base pairs (L).
4. The amount of formamide in the reaction mixture (F).

These variables have been linked by the following equation (see Meinkoth and Wahl, 1984): -

$$Tm = 81.5°C + 16.6 \log M + 0.41 (\% G+C) - 0.61 (\% F) - 500/L$$

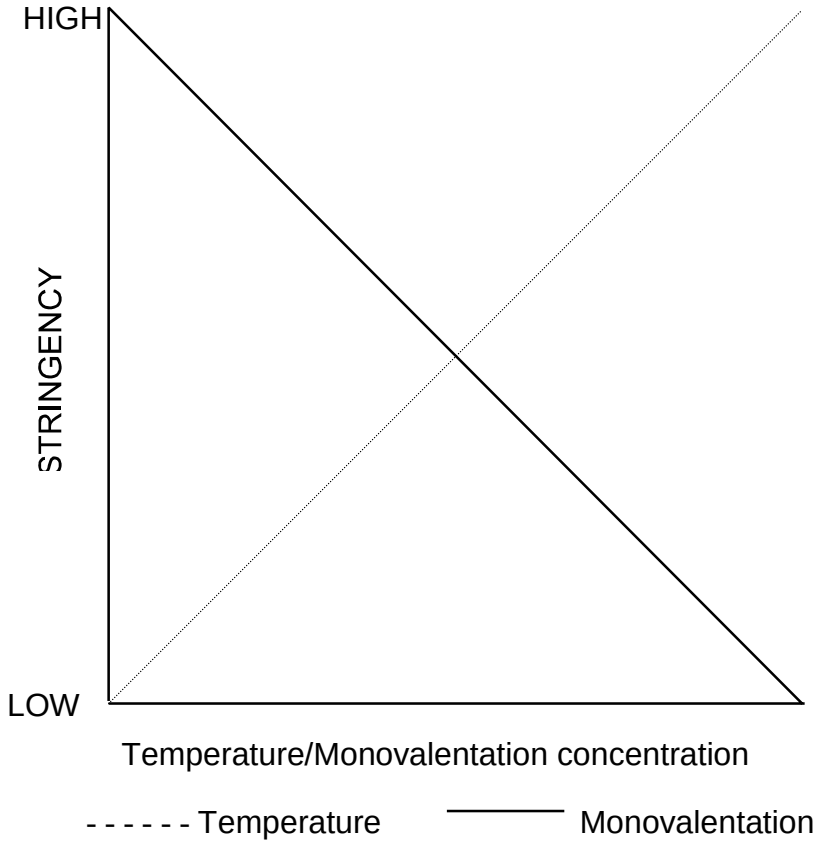
This equation relates to probes longer than approximately 50 nucleotides (see Chapter 6 for conditions relating to oligonucleotide Hybridisations).

As an illustration, in a reaction carried out in a solution of 6 x SSC and no formamide, with a 50% GC rich 500bp probe, the Tm is calculated to be 101°C (i.e. Hybridisation temperature 75°C).

Substituting the 6 x SSC with 1 x SSX in this system (i.e. during washing), reduces the Tm by 13°C. Including 50% formamide in each solution would have the effect of reducing each Tm by 30.5°C. A shorter probe length (50bp) reduces the Tm by a further 10°C.

In practice, it is not necessary to calculate the Tm accurately for every system, and the standard conditions given in the protocol sections will give good results. However, the equation does give a useful indication of the effect of adjusting the different variables on the stringency. In general, hybridise at low stringency; then during washing, increase the stringency by keeping the temperature constant but decreasing the salt concentration with each wash step.

Effect of Temperature & Salt Concentration



Summary of Conditions Affecting Hybridisation

Temperature

The Hybridisation temperature is one of the most critical factors because it affects the rate of hydrogen bond formation between DNA strands; the hydrogen bonds in effect overcome the repulsive forces between the negatively charged strands. If the temperature is increased, then the stringency of Hybridisation is increased.

Ionic Strength

Increasing the monovalent cation concentration enables the DNA strands to come together more readily, thus reducing the stringency of Hybridisation. EDTA is sometimes added to chelate magnesium ions, which can have an unpredictable effect on Hybridisation reactions.

Base Composition

Three hydrogen bonds are formed during G-C base pairing. G-C rich probes bind more efficiently, but may tend to bind non-specifically. Therefore, probes should have a G-C content of approximately 50-60%.

Formamide

Formamide acts as a destabilising agent, which reduces hydrogen bond formation. Hybridisation reactions using formamide are therefore performed at lower temperatures.

Probe Length & Complexity

A long complex probe will hybridise more slowly than a short probe.

High Molecular Weight Polymers, e.g. Dextran Sulphate

Dextran sulphate acts as an accelerator. In effect, it excludes the probe itself from a large volume of the probe solution effectively increasing local probe concentration. It also acts to some degree as a blocking agent.

Blocking Agents

For example, sonicated salmon sperm DNA, Denhardt's reagent. Blocking agents act as analogues to the probe, binding to regions in the filter that the DNA would otherwise tend to bind to non-specifically.

Detergents

Sodium dodecyl sulphate has the effect of increasing the stringency of Hybridisation, and can act as a blocking agent.

pH

For optimum results the pH of the Hybridisation solution should be maintained between pH 5-9.

Tetramethyl Ammonium Chloride (TMAC)

Generally speaking, A-T base pairs are less stable than G-C base pairs. TMAC, at a concentration of 3M, shifts the melting temperature of A-T base pairs to that of G-C base pairs. Thus it may be used in oligonucleotide Hybridisation so that the stringency of Hybridisation becomes a function of the probe length alone, rather than probe length and G-C content.

APPENDIX III
HYBRIDISATION BOTTLE SAFETY

Use of the Bottle Gripper

(Catalogue No HB-OV-BGS - to fit 15cm, small bottles)
(Catalogue No HB-OV-BGL - to fit 30cm, medium & large bottles)
(Catalogue No HB-OV-BGXL - to fit 30cm, extra large bottles)

This silicone rubber safety sleeve fits neatly over all our standard bottles and is included with every Thermo Oven we ship.

- Helping you to safely tighten and unscrew caps.
- Making handling of hot bottles easier.

  For more details and ordering information, contact Thermo Scientific or your local Distributor.

Bottle Care

Hybridisation Bottles are made of thick walled borosilicate glass, which protects users from radiation and has excellent long-term reliability.

- ☑ It is important to check your bottles regularly for chips, stress fractures and cracks. If these occur, the bottle must be discarded.
- ☑ Ensure bottles are stored either in a suitable rack or with caps replaced in between experiments. This will protect the bottle and sealing area.
- ☑ Replace “O” rings when worn or leaky. Replace all “O” rings every six months.
- ☑ Wear protective gloves to protect your hands in the event of accidental breakage.
- ☑ Never over tighten caps on bottles. Hand tight only is sufficient.
- ☑ If the bottle cap is difficult to unscrew **NEVER ATTEMPT** to force the bottle cap open. Allow the bottle to cool and retry. If the cap remains stuck, discard the bottle.
- ☑ The bottles should not be used at temperatures above 70°C.

NB: Extra large bottles (70mm diameter) are supplied with a vented cap to prevent excessive pressure build-up during use. A 0.45μm hydrophobic tissue culture filter should be fitted to the vent outside the bottle as an added safety measure.

APPENDIX IV
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Thermo Scientific
Controlled Environment Equipment
401 Millcreek Road
Marietta, Ohio 45750
United States

www.thermofisher.com